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Tischeriidae (Lepidoptera) leafminers new to Iran, including *Tischeria caucasica* on *Quercus*: a sibling species of *T. ekebladella* or a case of clinal variation?

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Abstract. We record three Tischeriidae species new for Iran: *Coptotriche gaunacella* (Duponchel, 1843) from Tehran and Mazandaran provinces, *Tischeria dodonaea* Stainton, 1858 from East Azarbaijan province and *T. caucasica* Klasiński & Stonis, 2020, previously only known from Georgia. The larvae of *T. caucasica* were observed mining the leaves of planted trees of *Quercus infectoria* G. Olivier, *Q. robur* Linnaeus and *Q. libani* G. Olivier in Peykan Shahr, Tehran province; and on native trees of *Q. castaneifolia* C.A.Mey. and *Q. macranthera* Fisch. & C.A.Mey. ex Hohen. in East Azarbaijan, Gilan and Mazandaran provinces. This species was very abundant on planted oaks in Peykan Shahr, Tehran and the infestation increased progressively in the second generation of the moth in November and December. *Tischeria caucasica* is very similar to European *T. ekebladella* (Bjerkander, 1795) in external appearance and biology, both sharing the same DNA barcode. The only diagnostic character, in the male genitalia, are the spiny appendages of the juxta. We provide a brief diagnosis and describe the larvae, leafmines and pupae for the first time. We discuss whether the observed difference in the male genitalia supports separate specific status, or is the result of clinal variation of a single species.

Introduction

Tischeriidae Spuler, 1899, or trumpet moths, is a small family of leafmining micromoths, with 158 described species in six genera globally (Stonis and Solis 2020; Stonis et al. 2020, 2021a, b). The larvae are leafminers during all larval instars (Diškus and Puplesis 2003a, b) and form trumpet-shaped or irregular blotch mines (Davis 1998), explaining the common name. This family is the only representative of the superfamily Tischerioidea and is known from all continents, except Australia and Antarctica. They belong to the small group of the oldest non-ditrysian lineages of Lepidoptera, and are sister to part of the austral family Palaephatidae which is not apparently monophyletic (Regier et al. 2015). Tischeriidae are associated with angiosperm plants belonging to 18 families, with the

main hosts being the families Asteraceae, Fagaceae, Malvaceae, Rhamnaceae, Rosaceae, and Urticaceae (Diškus and Puplesis 2003b; Stonis et al. 2020). In the West Palaearctic the family is rather poorly represented compared to other regions by 16 species (Diškus and Puplesis 2003b; Karsholt and van Nieukerken 2017; Klasiński et al. 2020; Rennwald and Rodeland 2021).

The original genus *Tischeria* Zeller, 1839 has this century been gradually dismantled by removing species to the five recently established new and revised genera, *Coptotriche* Walsingham, 1890, *Astrotischeria* Puplesis & Diškus, 2003, *Paratischeria* Diškus & Stonis, 2017, *Dishkeya* Stonis, 2020 and *Manitischeria* Diškus & Stonis, 2021. The genus currently consists of 11 Palearctic species feeding on oaks and chestnut (*Quercus* and *Castanea*), and one on *Ulmus* (Xu et al. 2018) and one Nearctic species on *Quercus.* Seven more Nearctic and Neotropical species, several feeding on Rhamnaceae, are still placed in *Tischeria*, but will likely also be removed to other genera after close scrutiny. The genus *Tischeria* is characterized in the male genitalia by long and slender uncus lobes, a slender and simple valva and by the well-sclerotized juxta, separated from the phallus; and in the female genitalia by the presence of a sclerotized antrum in the vestibulum (Stonis et al. 2021a). In the western Palaearctic four species of *Tischeria* are known, including the recently described *T. caucasica*, and all are leafminers on oaks (*Quercus*). Earlier descriptions and illustrations of *Tischeria ekebladella* and other European *Tischeria* species were published by Zagulyaev (1978), Diškus and Puplesis (2003b), Bengtsson et al. (2008), Klasiński et al. (2020), and the larva of *T. ekebladella* has extensively been described under the name *T. complanella* (Hübner, 1817) by Grandi (1929, 1931, 1933). The pheromones of female *T. ekebladella* were described by Molnár et al. (2012) and shown to attract the males.

So far, two Tischeriidae species have been reported from Iran: *Coptotriche angusticollella* (Duponchel, 1843) by Nematollahi and Radjabi (2002), and *C. marginea* (Haworth, 1828) by Christoph (1873) and Mirzayans and Kalali (1970), under the names of *Tischeria angusticollella* Duponchel and *Tischeria marginea* Haworth, respectively.

Recently some signs of damage by a leafminer on leaves of various oak trees were detected in the National Botanical Garden of Iran in Peykan Shahr, Tehran province. The National Botanical Garden of Iran with an area of 145 hectares, is located on the southern slopes of the Central Elburz Mts and includes various habitats categorised as Hyrcanian forests, Zagros, Irano-Turan region, Alborz, Caucasus, Europe, USA, China and Japan, etc. This botanic garden was founded in 1969 and most of its oak trees are concentrated in the Hyrcanian forests and Zagros habitats (Jamzad et al. 2019). The oak trees in this area were infested with rather small, round and almost pure white leafmines. Reared adults appeared to be very similar to *Tischeria ekebladella* (Bjerkander, 1795), based on external morphology, leafmine shape and COI sequences. However, examining the male genitalia, surprisingly, revealed a considerable difference with the latter species in the structure of the juxta. In fact, the recently described species *T. caucasica* Klasiński & Stonis, 2020 (in Klasiński et al. 2020) from Georgia, shows exactly the same male genitalia. Additional specimens of this species were collected in the East Azarbaijan, Gilan and Mazandaran provinces. Here we will discuss whether our specimens belong indeed to *T. caucasica*, and discuss DNA barcodes of *T. ekebladella* and *T. caucasica* and their variation. We diagnose the species and describe the larvae, pupae and biology of this species for the first time.

In 2007 JB and HA collected leafmining Lepidoptera, mainly Gracillariidae, in northern Iran. As a by-product a few Tischeriidae were reared, and we cite here those novel records, including two more species new for Iran.

Material and methods

In 2007 and 2009 leafmines with larvae were collected during field trips in the provinces of East Azarbaijan, Mazandaran, and Tehran. Larvae were reared in the laboratory in Poland. Moths in collections from these expeditions are divided between HMIM and RMNH.

In the National Botanical Garden of Iran, Peykan Shahr, Tehran, leaves with mining larvae and cocoons were sampled between June and November 2016 and 2018. They were reared in plastic jars containing wet cotton under laboratory conditions in Tehran.

Moreover, specimens were borrowed from RMNH and ZMUC by HA and compared with the Iranian specimens. Genitalia were prepared following the standard method of Robinson (1976). Genitalia and abdomen were stained with Chlorazol Black and mounted on slides in Euparal; in Leiden, some males were stained with Phenosafranine. For descriptive terminology we refer to Diškus and Puplesis (2003b) and Stonis et al. (2020) for adults and Grandi (1933) for larvae.

Photographs of larvae, pupae and genitalia structures were taken using a digital camera DSC-F717 and a Dino-Eye microscope eye-piece camera AM423X. The software Combine ZP was also used to stack some images. In Leiden adult moths were photographed with an AxioCam MSc camera attached to a Zeiss Stemi-SV11 using AxioVision software; genitalia were photographed with the same camera attached to a Zeiss Axioskop compound microscope. Some images of moths were stacked with Zerene Stacker software.

Collected larvae were preserved in 70% ethanol. Measurements of head capsules were made across the greatest width of the head (genae) under a stereomicroscope with an eyepiece micrometer. Means and standard deviations were calculated for each instar. Determination of the number of larval instars was based on Dyar's Rule (Dyar 1890).

Abbreviations

HMIM Hayk Mirzayans Insect Museum, Teheran, Iran
RMNH Naturalis Biodiversity Center, Leiden, the Netherlands
ZMUC Zoological Museum, University of Copenhagen, Denmark

DNA extraction and sequencing

DNA was extracted non-destructively from the abdomens of two adult specimens sent to Leiden, while at the same time the genitalia could be prepared; larvae from Georgian specimens were dissected out of dried leaves and DNA was extracted from one of these also non-destructively; larval remains were prepared on slide as well. Laboratory protocols follow Doorenweerd et al. (2016). The voucher and sequence data are available on BOLD (Ratnasingham and Hebert 2007) in the public dataset DS-TISCIRAN (dx.doi.org/10.5883/DS-TISCIRAN), including specimens of other European *Tischeria* species, partly obtained from third parties. We provide Barcoding Identification Numbers (BIN) for each species (Ratnasingham and Hebert 2013). All COI sequences were also deposited in GenBank, details of which can be found in the cited BOLD dataset. We used tools from BOLD for preparing the Neighbor Joining tree (Fig. 11) with the Kimura 2 Parameter as Distance Model.

Results

Tischeria caucasica Klasiński & Stonis, 2020

Diagnostic remarks. *Tischeria caucasica* is very similar to *T. ekebladella* (Fig. 1A–C). However, as stated by Klasiński et al. (2020), males can easily be distinguished from *T. ekebladella* and *T. ekebladoides* Puplesis & Diskus, 2003 by the unique, very long and distally bifurcate appendages of the juxta (Fig. 2A–H).

In the dissected males of *T. caucasica* from Iran, there is some variation in the shape and length of the horn-like appendages of the juxta. Based on the results of this study, the apex of the longer pair of juxtal appendages is not always bifurcated. In some of the examined males, both appendages have bifurcated tips (Fig. 2A, F); while in some others the shorter one has simple and longer one has bifurcated tip (Fig. 2B, D, E). This has also been illustrated by Klasiński et al. (2020: 15, figs 15, 21–23), although without any explanation of the variation. Moreover, as we revealed in this study, the longest appendage of the juxta if bifurcated apically, the two branches are either assymetrical or symmetrical (Fig. 2A, B, D, F).

According to Klasiński et al. (2020), the two very long horn-like processes of juxta are bent basally; however, as we discovered in this study, the shorter one is always bent at its distal two-thirds, but the longer process is usually curved internally (Fig. 2A–F). Additionally, the anterior tip of the triangular ventral plate of vinculum varies from narrow (Fig. 2A) to relatively wide (Fig. 2B, D).

In the genitalia of examined females, as described by Klasiński et al. (2020), the antrum has a weakly sclerotized anterior margin with a unique membranous part, and the shortest prela has a wide base (Fig. 3A–D); however, the corpus bursae is not always heavily folded longitudinally (Fig. 3A).

No external difference between *T. caucasica* and its closely related species was reported by Klasiński et al. (2020). Based on the results of this study, *T. caucasica* is close to both *T. ekebladella* and *T. ekebladoides* in wing pattern and size. However, there are a few differences between *T. caucasia* and *T. ekebladella* (Fig. 1A–C, E–G): 1) *T. caucasica* has a partly less pointed hindwing compared with *T. ekebladella*; 2) Fringes of the hindwing in *T. caucasica* are slightly paler than in *T. ekebladella*; 3) Ventro-apical fringes of forewing in *T. ekebladella* are slightly darker than in *T. caucasica* and 4) On the underside of the forewing, the costal area at the apex is slightly paler in *T. caucasica* compared with that of *T. ekebladella*.

Tischeria caucasica differs from *T. ekebladoides* in the following external features (Fig. 1A, B, D): 1) *T. caucasica* has a wider and less pointed hindwing compared with *T. ekebladoides*; 2) The ground colour of the body and forewing in *T. caucasica* are darker than in *T. ekebladoides*; 3) In *T. ekebladoides* the frontal tuft and collar area have scattered brown scales and 4) The forewing of *T. ekebladoides* is slightly narrower than that of *T. caucasica*.

Based on the results of this study, the female of Iranian *T. caucasica* population has a slightly longer forewing than the male (Fig. 1A, B), with no significant difference between the two generations. Forewing length in the first generation was 3.5–4.6 mm ($x = 4.11$ mm $\pm$ 0.26, $n = 44$) in the male and 3.6–4.9 mm ($x = 4.37$ mm $\pm$ 0.26, $n = 49$) in the female. In the second generation it was 4.0–4.4 mm ($x = 4.12$ mm $\pm$ 0.16, $n = 8$) in the male, and 4.0–4.7 mm ($x = 4.31$ mm $\pm$ 0.25, $n = 7$) in the female.

Leafmines of *T. caucasica* cannot be separated from those of *T. ekebladella*. Moreover, the larvae of these two species are indistinguishable externally.

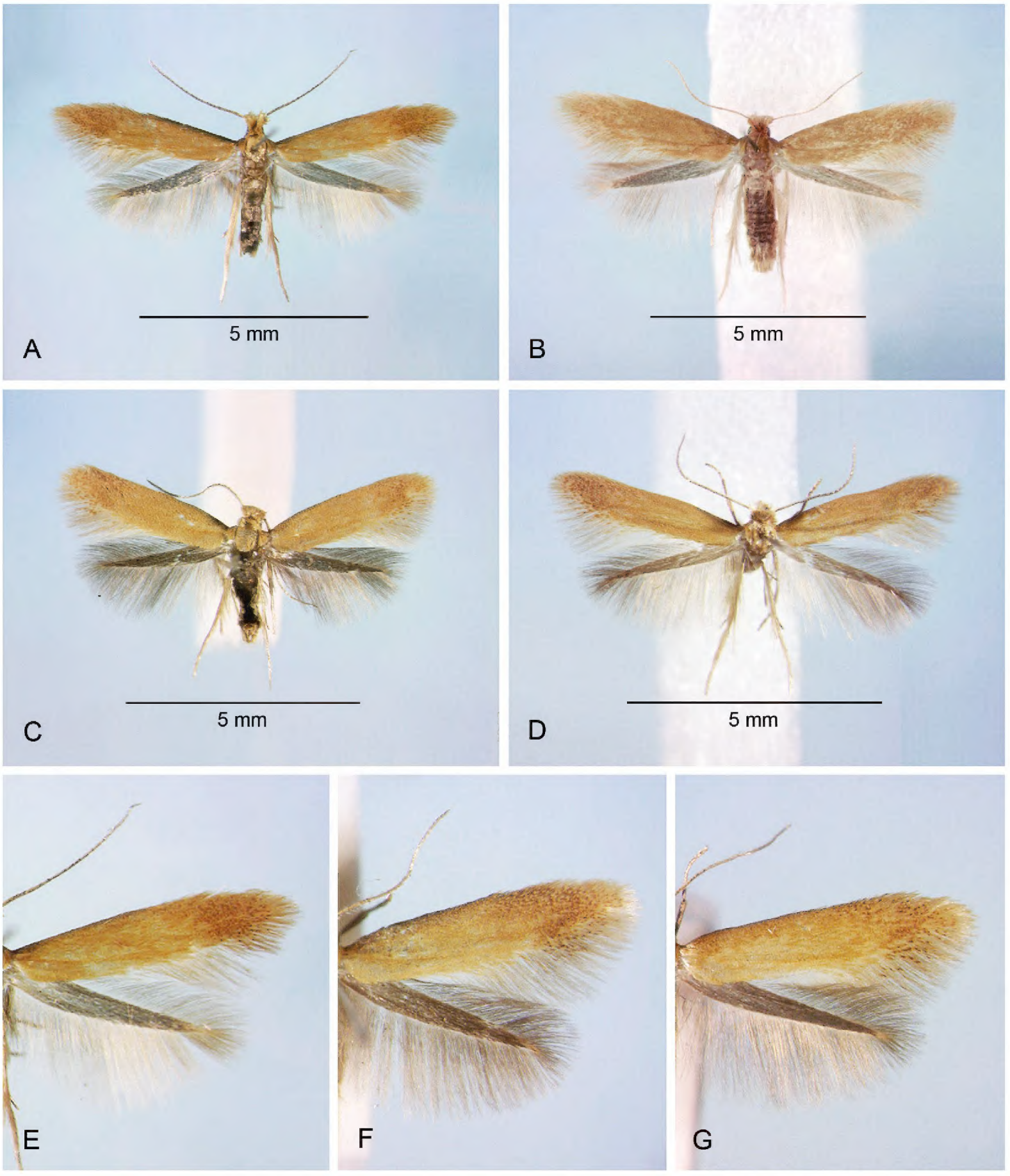

Figure 1. *Tischeria* spp. adults, dorsal view. **A.** *T. caucasica*, male, Tehran (Peykan Shahr); **B.** *T. caucasica*, female, Tehran (Peykan Shahr); **C.** *T. ekebladella*, male, France; **D.** *T. ekebladoides*, male, paratype, Spain (Andalusia); **E–G.** Right wings of *T. caucasica*, male (**E**), *T. ekebladella*, male, Netherlands (Limburg) (**F**) and *T. ekebladella*, male, Belgium (Namur) (**G**).

DNA Barcoding. A Neighbor Joining tree is provided for barcodes of *T. caucasica* and *T. ekebladella*, together with barcodes of the other European species *T. dodonaea* Stainton, 1858 and *T. decidua* Wocke, 1876 (Fig. 11). Each species has its own BIN, and is recognizable by its DNA barcode, except the pair *T. caucasica* and *T. ekebladella*. Unfortunately no barcodes are available for *T. ekebladoides*.

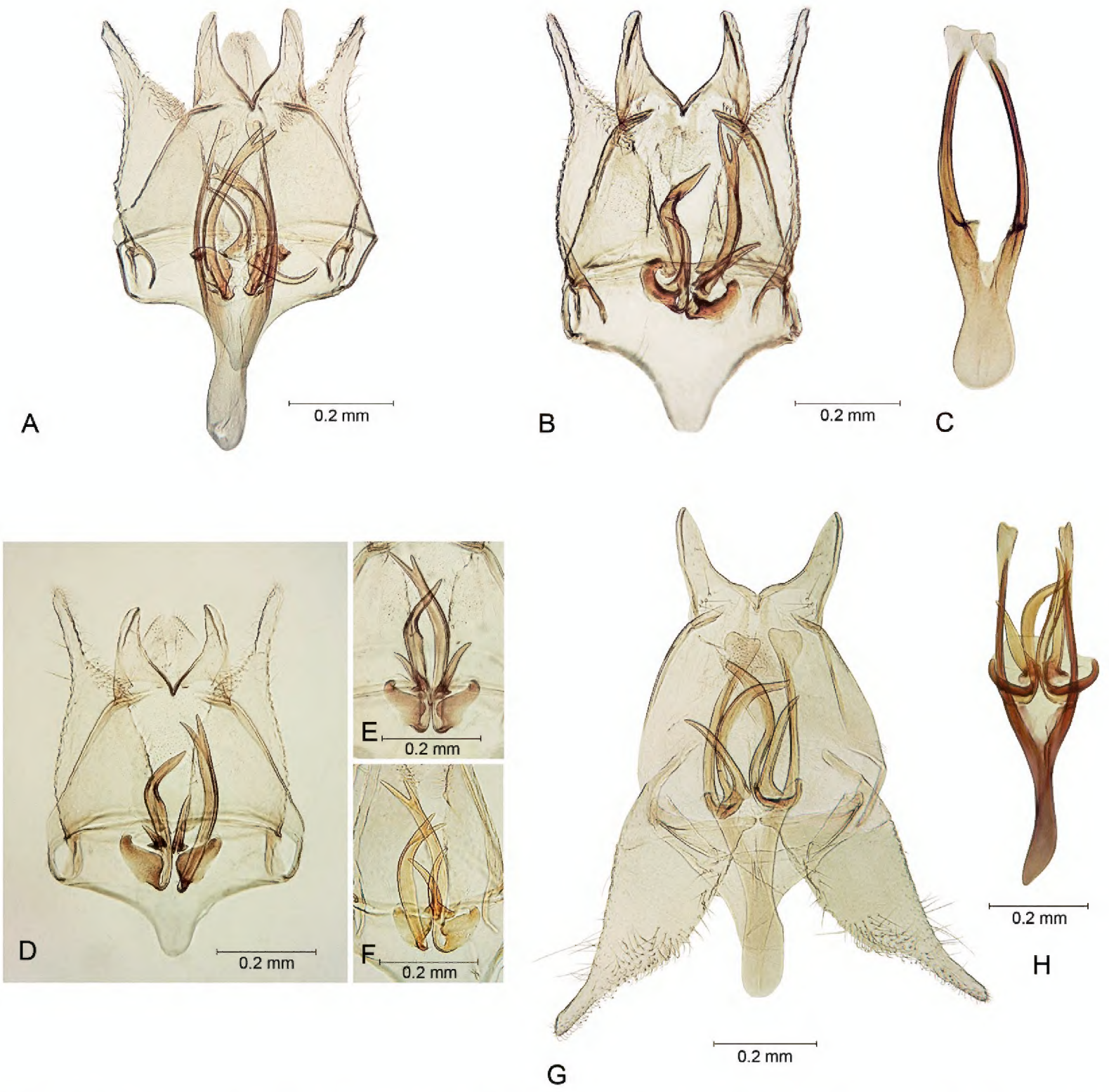

Figure 2. Male genitalia of *Tischeria* spp. **A–F.** *T. caucasica* (Tehran, Peykan Shahr): phallus in situ (**A**) (genitalia slide HA-2627, HMIM), phallus removed (**B, D**) (genitalia slides HA-2378, HA-2628, HMIM) (in figure **D** one of the shortest appendages of juxta has been bent backward during slide preparation and makes it look much shorter than the other one), phallus (**C**) (genitalia slide HA-2378, HMIM), juxta (**E, F**) (genitalia slides HA-2626, HA-2625, HMIM); **G, H.** *T. ekebladella* (Netherlands): phallus in situ (**G**), phallus and juxta (**H**) (genitalia slide JCK752).

DNA barcodes of two Iranian specimens appeared to be identical to that of a Dutch specimen of *Tischeria ekebladella* and fall within one BIN BOLD:AAF8247, that includes also all other available *T. ekebladella* barcodes. The barcode of a Georgian larva from leafmines examined by EvN (RMNH.INS.31425) groups with the Georgian adult of *T. caucasica* (GBRD286), together as sister to all remaining *T. ekebladella* and somewhat different from the Iranian *T. caucasica*. Within BIN BOLD:AAF8247 the average distance is 0.38% and the maximum distance is 1.93% (n = 54). The nearest neighbor is BOLD:ACU6278, representing the East Palearctic *Tischeria siorkionla* Kozlov, 1986, with a distance of 8.01%.

Figure 3. Female genitalia of *Tischeria caucasica* (Tehran, Peykan Shahr). **A, B.** Main body in ventral view (genitalia slides HA-2376, HA-2379, HMIM); **C.** Detail of terminalia (genitalia slide HA-2376, HMIM); **D.** Detail of antrum area (genitalia slide HA-2377, HMIM).

Description of immatures. Last instar larva (Fig. 4A–I) pale yellowish green, with pale light-brown head, pronotal and anal plates slightly darker than body. Head flattened with almost 5–6 stemmata arranged linearly, two posterior ones with a relatively larger distance to the remaining ones (Fig. 4C). Hairs and bristles distributed as in *T. ekebladella* (for comparisons with that species refer to Grandi 1929, 1931, 1933, which are virtual identical descriptions).

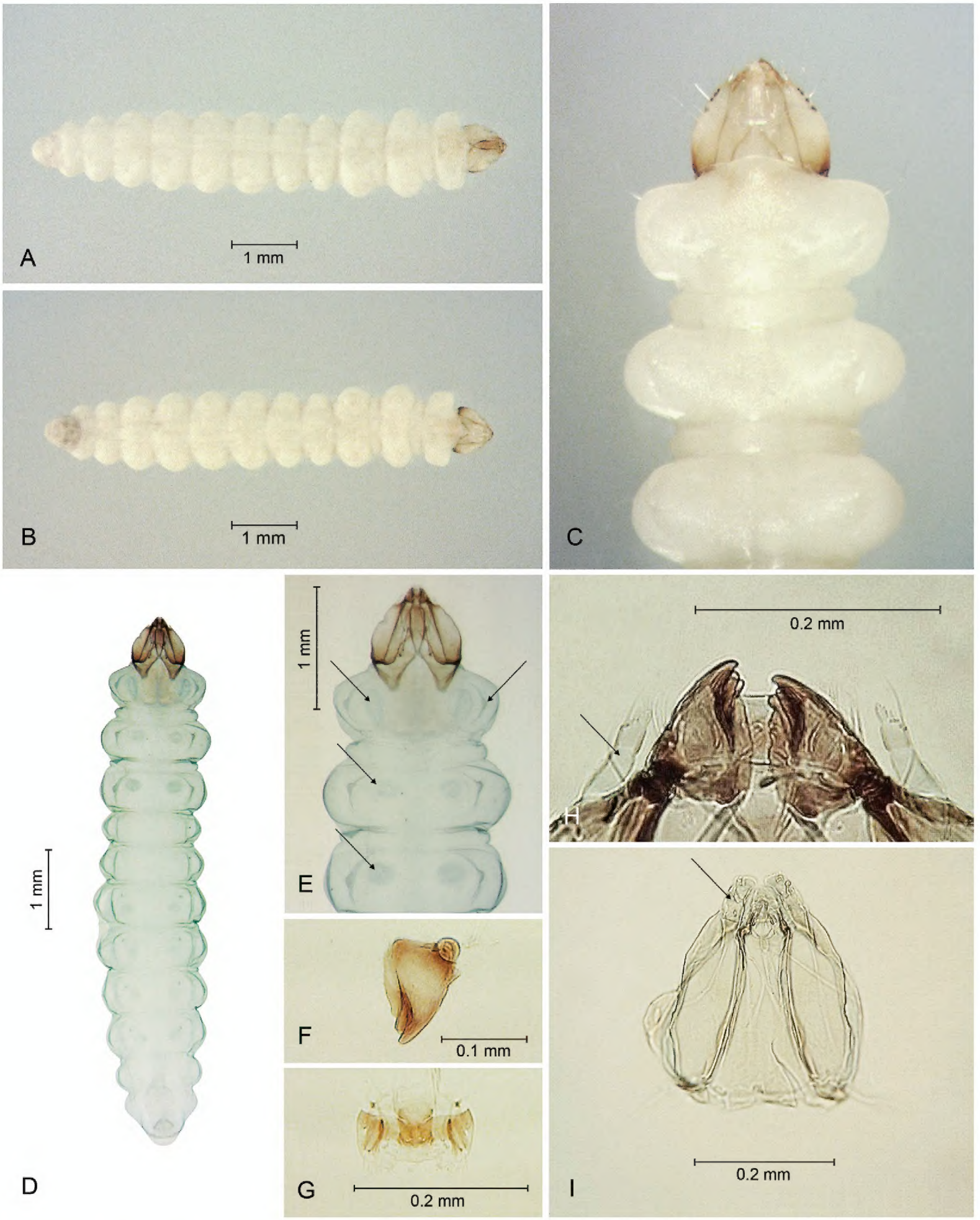

Figure 4. Last instar larva of *Tischeria caucasica* (Tehran, Peykan Shahr). **A, B, D.** Larva in dorsal (**A, D**) and ventral (**B**) views; **C, E.** Anterior part in ventral (**C**) and dorsal (**E**) views including head and thoracic segments (the upper, median and lower arrows in the left side of figure **E** indicate the slightly sclerotized plates at pro-, meso- and methathorax; the arrow in the right side indicates the thoracic leg which is visible from dorsal surface); **F.** Mandible; **G.** Labrum; **H.** Anterior part of the head in dorsal view showing antenna (arrow) and mandibles; **I.** Maxilla (arrow), maxillary palpi and labium.

Labrum (Fig. 4G) rectangular, and trilobed distally, with the two lateral lobes protruded considerably beyond median one, and a pair of fronto-lateral bristles; lateral and sub-posterior elements and medial ones represented by very small hairs, barely visible at very strong magnification. Mandible (Fig. 4F, H) sub-triangular, slightly longer than wide, tridentate apically with blunt tips; oral margin of mandible at proximal half with some setiform processes bent backwards and of different lengths (only visible under very large magnification). Maxillae (Fig. 4I) as in *T. ekebladella*, with large laminar stems provided with two very minute placoid structures, its detailed structure hardly visible under available magnification. Maxillary palpi (Fig. 4I) composed of three articles, decreasing in width towards the apex. Labium (Fig. 4I) nearly as that of *T. ekebladella*, but bi-articulated labial palpi were hardly visible under the available maximum magnification.

Antennae (Fig. 4H) three-segmented, segment 1 sub-cylindrical, slightly less than twice as long as wide, segment 2 slightly longer than wide, slightly less than half length of segment 1; distally with a long bristle, bristles on antennal surface hardly distinguishable under available magnification.

Thorax and abdomen. Nearly as in *T. ekebladella*; however, first thoracic segment not wider than other two (Fig. 4A–E). In the examined larvae the 2nd and 3rd thoracic segments mostly with same width and length (although in some specimens segment 2 is slightly wider than 3), segment 1 always the shortest, its width slightly less than two remaining thoracic segments or occasionally with same width (Fig. 4A–E). Abdominal segment 1 normally shorter than segments 2–6 (Fig. 4A, B), but in some specimens segments 1 and 2 equal in length and shorter than segments 3–6 (Fig. 4D). Abdominal segments 3–6 and sometimes 2–6 are usually the longest. Abdominal segment 1 usually slightly narrower than 2–6 or 3–6 (Fig. 4A, B, D).

Thoracic segments each with pair of very slightly sclerotized circular to elliptical plates on both dorsal and ventral surfaces towards the lateral sides (Figs 4D, E, 5A, B, F). The plates of the 2nd and 3rd tergites and sternites have nearly the same size and shape, and if elliptical positioned transversely. The plates of the 1st tergite are elongate and somehow obliquely arranged to the longitudinal axis of the body (Figs 4D, E, 5A); while those of the 1st sternite are sometimes irregularly shaped (Fig. 5F). These plates usually have a less sclerotized centre and are hardly visible, but they can be observed after staining the abdomen (Figs 4D, E, 5A, B, F).

Thoracic segments with single long bristle laterally, and pair of short bristles dorso-laterally (Fig. 5B). Thoracic legs nearly pyramid-shaped or finger-shaped (Figs 4D, E, 5F), very short, bearing two microscopic internal hairs distally (Fig. 5F).

Abdominal tergites 1–8 with pair of long bristles on either side. Tergites 1 and 2 with two pairs of short bristles positioned rather medio-laterally, and nearly in a line (Fig. 5C). Tergites 3–6 with two pairs of short bristles, which are not in the same line and positioned more laterally, and two pairs of nearly short, robust asymmetrical bristles internal to the lateral pairs, implanted on a common base or with bases completely confluent (Fig. 5D, E). Tergites 7 and 8 each with a submedial hair. Tergite 8 with five pairs of long marginal hairs, and some serration towards the middle part. Four pairs of the prolegs on segments 3–6 atrophied, without any trace of crochets (Figs 4D, 5G), but with three hairs externally (Fig. 5G). Last proleg provided with pair of linear series of crochets, which in specimens examined each contain between 20–24 elements. They are not in equal numbers normally (n = 3) (Fig. 5H). For measurements of head capsule and body see Table 1.

Pupa (Fig. 6A–E) pale ochre to ochreous-brown, with brown scales at the dorsal surface of abdominal segments. The sex of the pupa can be easily recognised by shape of last abdominal segment (Fig. 6D, E). In the female, the paired triangular processes at the end of last abdominal tergum

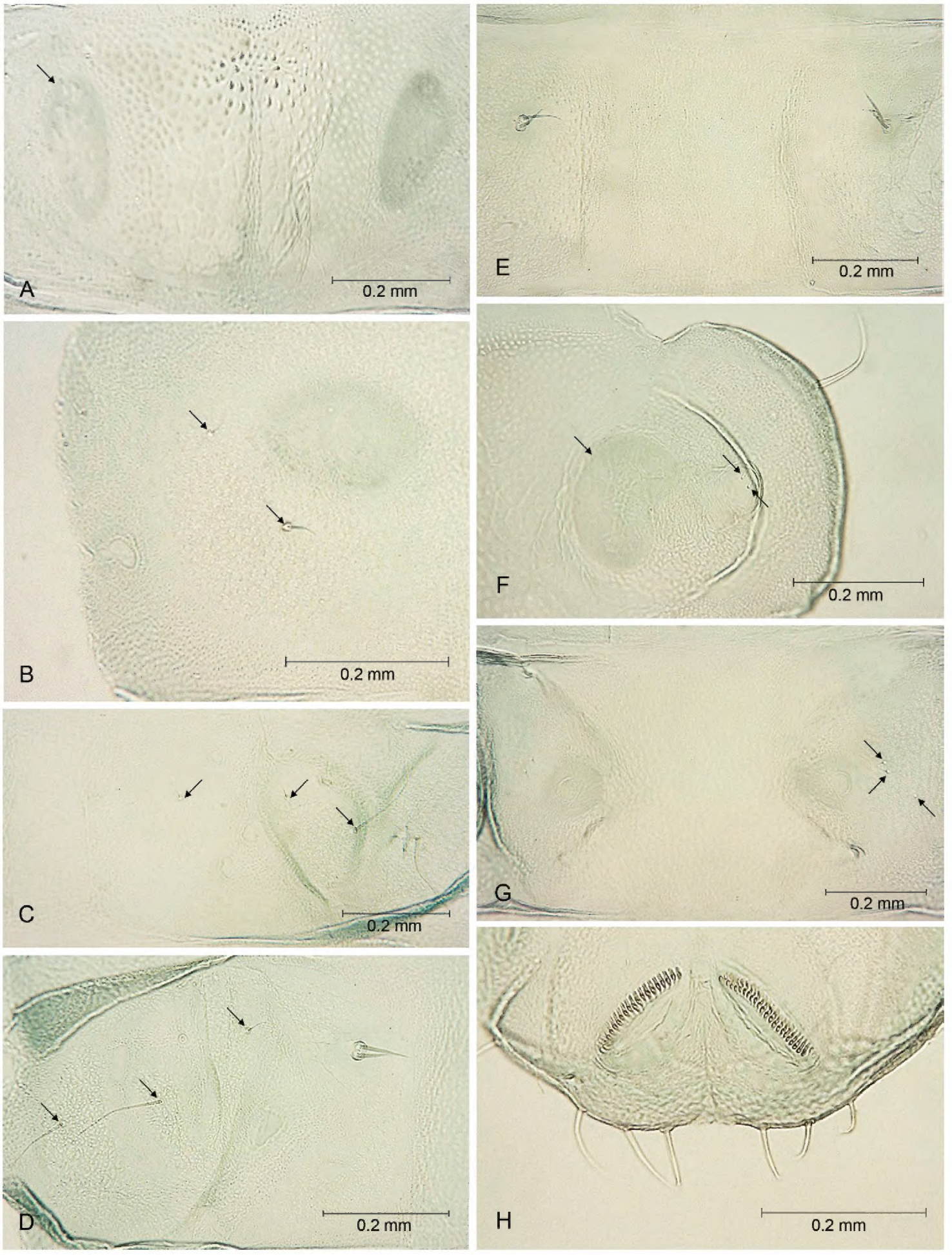

Figure 5. Terga and sterna of the last larval instar of *Tischeria caucasica* (Tehran, Peykan Shahr). **A.** Prothorax (arrow indicates the slightly sclerotized plate); **B.** Metathorax, left half (arrows showing the bristles on dorsal and dorso-lateral sides); **C.** Second abdominal segment in half view (arrows indicate the bristles on medio-lateral and lateral sides); **D, E.** Third abdominal segment in half view (**D**) and median part (**E**) (arrows indicate arrangement of the bristles on dorsal and lateral sides); **F.** Ventral surface of first thoracic segment in half view showing slightly sclerotized plate (left arrow) and first thoracic leg (arrows indicate the microscopic hairs at the distal end of the leg); **G.** Ventral surface of the third abdominal segment showing the atrophied prolegs (arrows indicate the hairs positioned external to proleg); **H.** Pair of linear series of hooks in the last proleg.

Table 1. Head capsule width and body length (min-max; mean ± SE, mm) of different larval instars in *Tischeria caucasica*.

Larval instar	n	Min-Max	Width (mm)	Min-Max	Body length (mm)
1	15	0.19–0.27	0.23 ± 0.02	0.76–1.48	1.07 ± 0.22
2	11	0.28–0.37	0.32 ± 0.03	1.41–3.35	1.96 ± 0.56
3	20	0.41–0.47	0.44 ± 0.02	2.03–4.20	3.56 ± 0.49
4	51	0.52–0.69	0.61 ± 0.03	3.25–6.60	5.29 ± 0.85

are slightly larger and closer to each other than in the male (Fig. 6D). Papillae anales of female genitalia usually visible. Female pupa slightly larger than male. Length of male pupa 3.70–4.40 mm (x = 4.06 mm ± 0.17, n = 22); female pupa 3.50–5.1 mm (x = 4.28 mm ± 0.43, n = 25).

Biology. Host plants: In Iran *Quercus castaneifolia*, *Q. infectoria*, *Q. libani*, *Q. macranthera*, and *Q. robur* (Fagaceae). In Peykan Shahr the percentage of infection in *Q. robur* was very high (Fig. 7B–I), higher than in *Q. infectoria* (Fig. 8A–C), and in the latter species it was higher than in *Q. libani* (Fig. 8D, E). In *Q. libani* the infection rate was very low (one or two mines on each leaf) (Fig. 8D). Mines from Georgia (one larva barcoded RMNH.INS.31425) that most likely belong to this species, were found on *Q. petraea* subsp. *polycarpa* (Schur) Soó and *Castanea sativa* Mill.

Based on the results of this study, *T. caucasica* has two generations per year in Tehran province. The eggs are white-grey in colour, deposited on the upper side of the leaf near the mid-rib or on larger lateral ribs. The mines consist of milky white upper-surface primary flat blotches, sometimes with orange hues, and like *T. ekebladella*, with no traces of preceding corridors (Figs 7A–K, 8A–E). As they get older, they turn very pale brown (Figs 7A–H, 8A–C). The cocoon was made nearly at the centre of each blotch (the so called nidus) and pupation took place within the mine. No frass was observed inside the mine (Figs 7G, K, 8B, C).

Leafmines were observed in 2017 and 2018 from late May to the first half of December, when the blotches were very minute. The greatest number of the mines in the first generation appeared in the first half of June. Adults were observed in early to mid July, when the next generation would occur. In the first generation, many adults emerged and, as a result, the population density increased remarkably during the second generation. In November, during leaf fall, the last instar larvae were overwintering inside the blotches. Live larvae were observed, yet sedentary through a cut in the upper epidermis in winter at the height of the cold. They became active and pupated after the end of the cold weather period.

Four larval instars were estimated by measuring the width of the head capsule of each larval instar and applying Dyar's law (Dyar 1890). Body length and head capsule width, min-max (mean ± SE) in all larval instars, are shown in Table 1.

Seasonal development. In Tehran (Peykan Shahr) the activity period of *T. caucasica* larvae started from the end of May and continued in two generations until the beginning of December. In the first generation during 2018, the 1st larval instar hatched late May (22nd) and feeding on leaf tissue within the mine. The first and second larval instars formed a short linear mine towards the leaf edge. Third instar larvae appeared at the end of May (27th) , the last instar nearly at the first half of June (10th), and adults emerged from mid June (16th) to the 1st of July.

The number of mines per leaf varies; in some cases there are as many as 12 mines (Fig. 8C). The first larval instar of the second generation appeared at the end of August (30th) and the last instar on late September. They overwintered as 4th instar larvae and remained quiescent until pupation in late May to early June. According to the results of this study, *T. caucasica* had a severe outbreak

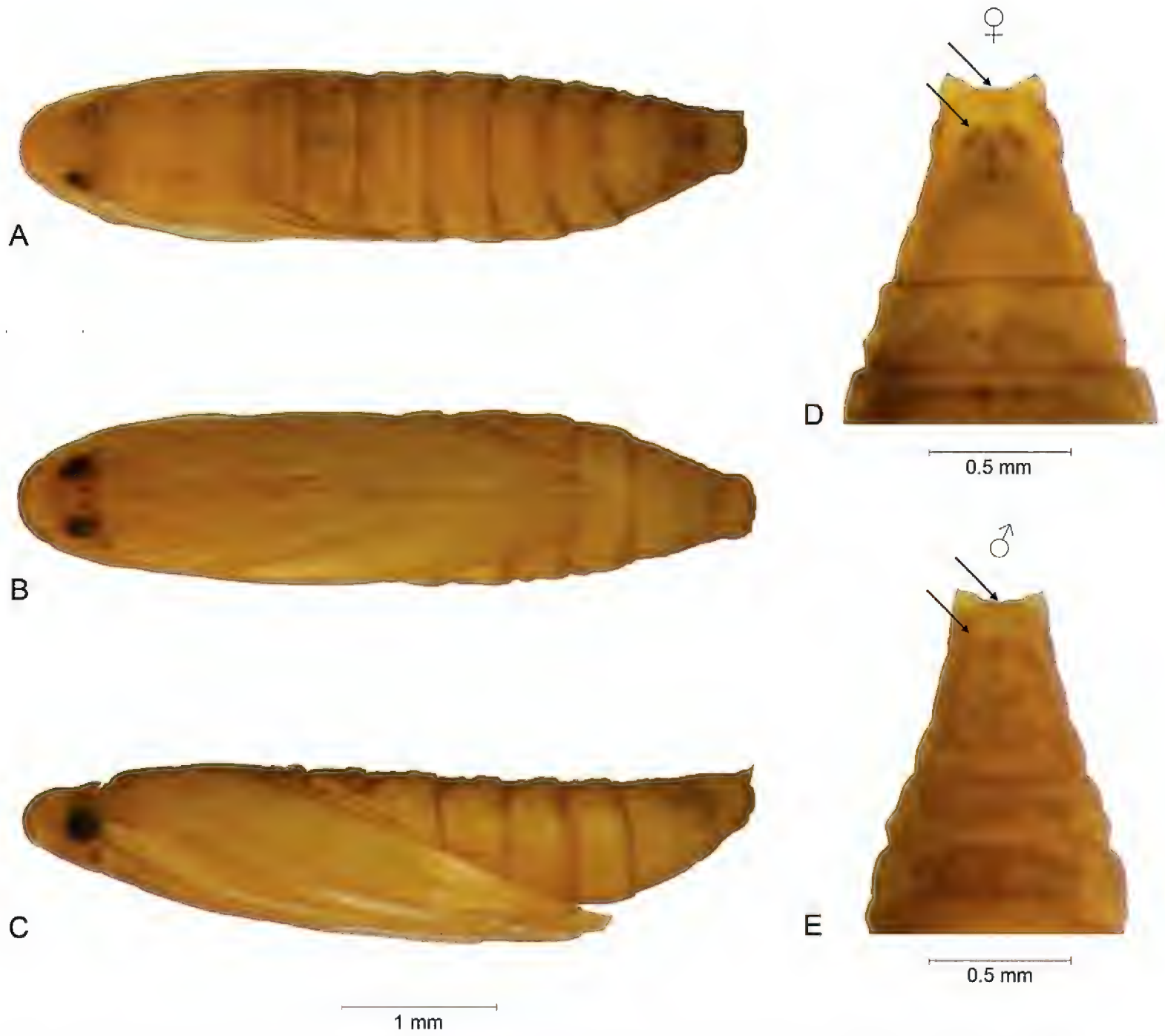

Figure 6. Pupae of *Tischeria caucasica* (Tehran, Peykan Shahr). **A–C.** Whole body in dorsal (**A**), ventral (**B**) and lateral (**C**) views; **D, E.** Posterior half of the body in female (**D**) and male (**E**) (arrows indicate pronounced differences between female and male pupae).

between 2017 to 2019, despite spraying the trees and destroying the fallen leaves. In 2020, the infection was considerably lower, and there was no trace of infection in the garden until May 2021.

Two species of parasitic wasps, *Brachymeria excarinata* Gahan, 1925 (family Chalcididae) and *Aprostocetus* sp. (family Eulophidae) emerged from the mines.

Distribution. Georgia (Klasiński et al. 2020) and Iran (Tehran: Peykan Shahr; East Azarbaijan: Ainalou; Gilan: Molumeh; Mazandaran: Neka) (Fig. 9).

Material examined. Iran, Tehrān Prov., Tehrān, Peykān Shahr, 35°44'27"N, 51°10'50"E, 1317 m, 33 ♂♂ 26 ♀♀, emerged 6, 9, 16, 19, 21, 23, 24.vi.2018, 11 ♂♂ 23 ♀♀, emerged 3, 6, 7, 10, 13.vii.2019 (first generation); 7 ♂♂ 8 ♀♀, emerged 9.xii.2018 (second generation), S. Farahāni leg., all reared from *Quercus robur* (genitalia slides HA-2375, HA-2376, HA-2377, HA-2378, HA-2379, HA-2380, HMIM); Same locality, 1 ♂, 1 ♀, emerged 11.vi.2016, genitalia slides EvN5029 (♂), EvN5030 (♀), specimens barcoded, RMNH.INS.25029, RMNH.INS.25030 (RMNH); 2♂, 1♀, not pinned, same data, RMNH.

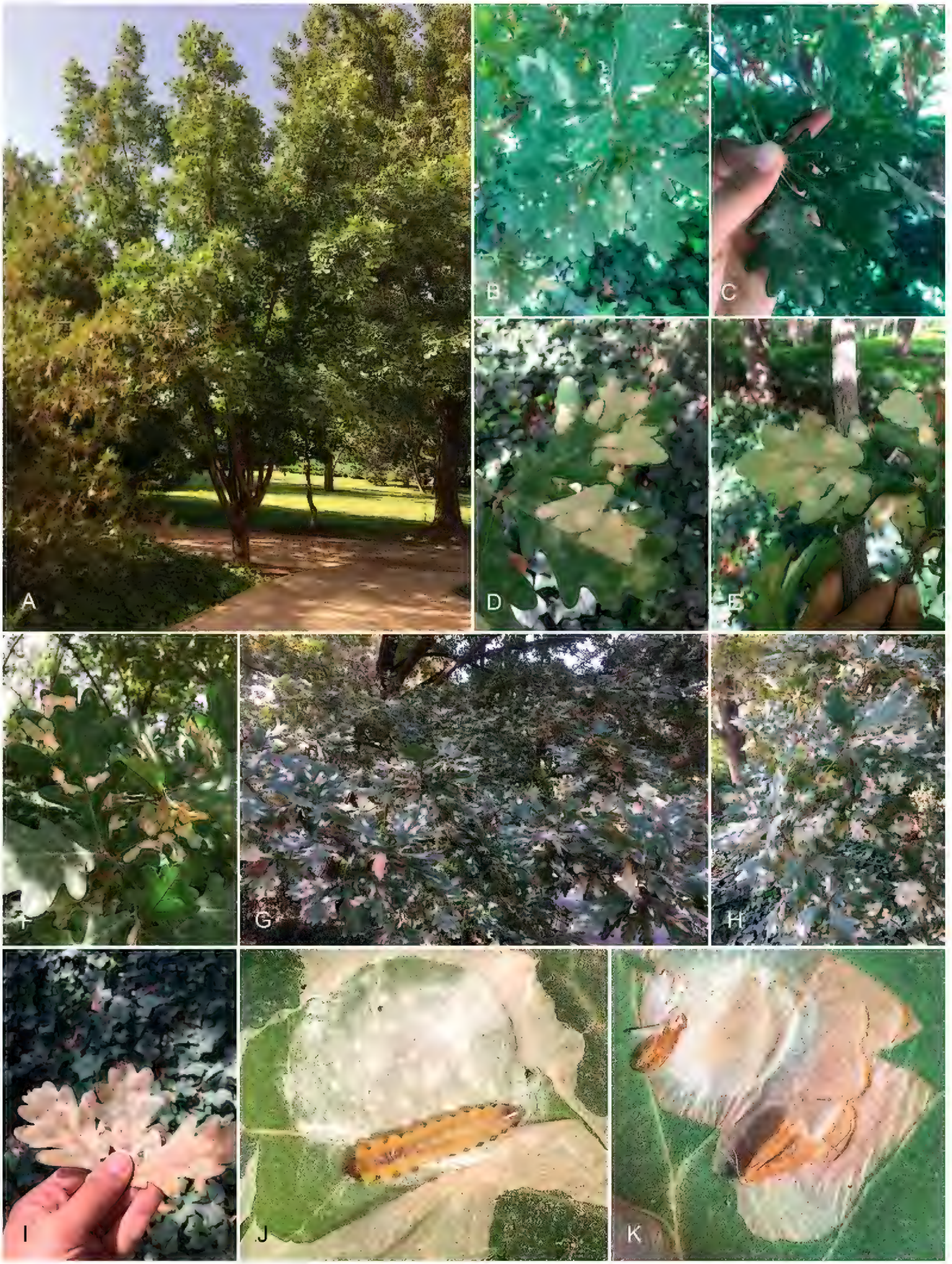

Figure 7. Blotch mines of *Tischeria caucasica* (Tehran, Peykan Shahr) on *Quercus robur*. **A.** infected tree; **B, C.** Young mines (first generation), early June, 2018; **D.** Full grown mines (first generation), early July, 2018; **E, F.** Developing mines (second generation), mid July, 2018; **G–I.** Old mines (second generation), end of September, 2018; **J.** Last instar larva dissected from the mine, second generation; **K.** Pupal exuviae and adult next to opened mine, second generation. The greatest number of mines in the first generation appeared in the first half of June. Adults were observed from early to mid July, when the next generation would occur.

Figure 8. Blotch mines of *Tischeria caucasica* (Tehran, Peykan Shahr) on *Quercus infectoria* (**A–C**), and *Q. libani* (**E, F**).

East Āzarbāijān Prov., Arasbārān protected area, Āsheghlou to Vāyeghān Rd., near Āinalou, 38°58'4.3"N, 46°42'27.6"E, 513 m, 17.ix.2008, J. Buszko leg., *Quercus macranthera*, 3 mines, 1 ♂ 2 ♀♀, emerged 16.–26.iii.2009, genitalia slide ♂ EvN5287, RMNH.INS.25287, RMNH, HMIM; Gilān Prov., Molumeh, 36°51'17.01"N, 49°55'48.60"E, 11.ix.2008, J. Buszko leg., *Quercus macranthera*, 3 mines, 1 ♂ 1 ♀, emerged 17.iii.2009, genitalia slide ♀ EvN5291, RMNH.INS.25291, RMNH, HMIM.

Māzandarān Prov., Nekā, 36°30'16.7"N, 53°23'27"E, 530 m, 30.ix.2007, J. Buszko leg., *Quercus castaneifolia*, 4 mines, 2 ♂♂ 2 ♀♀, emerged 20.–26.ii.2008, genitalia slides ♂ EvN5289, EvN5290, RMNH.INS.25289, RMNH.INS.25290, RMNH, HMIM.

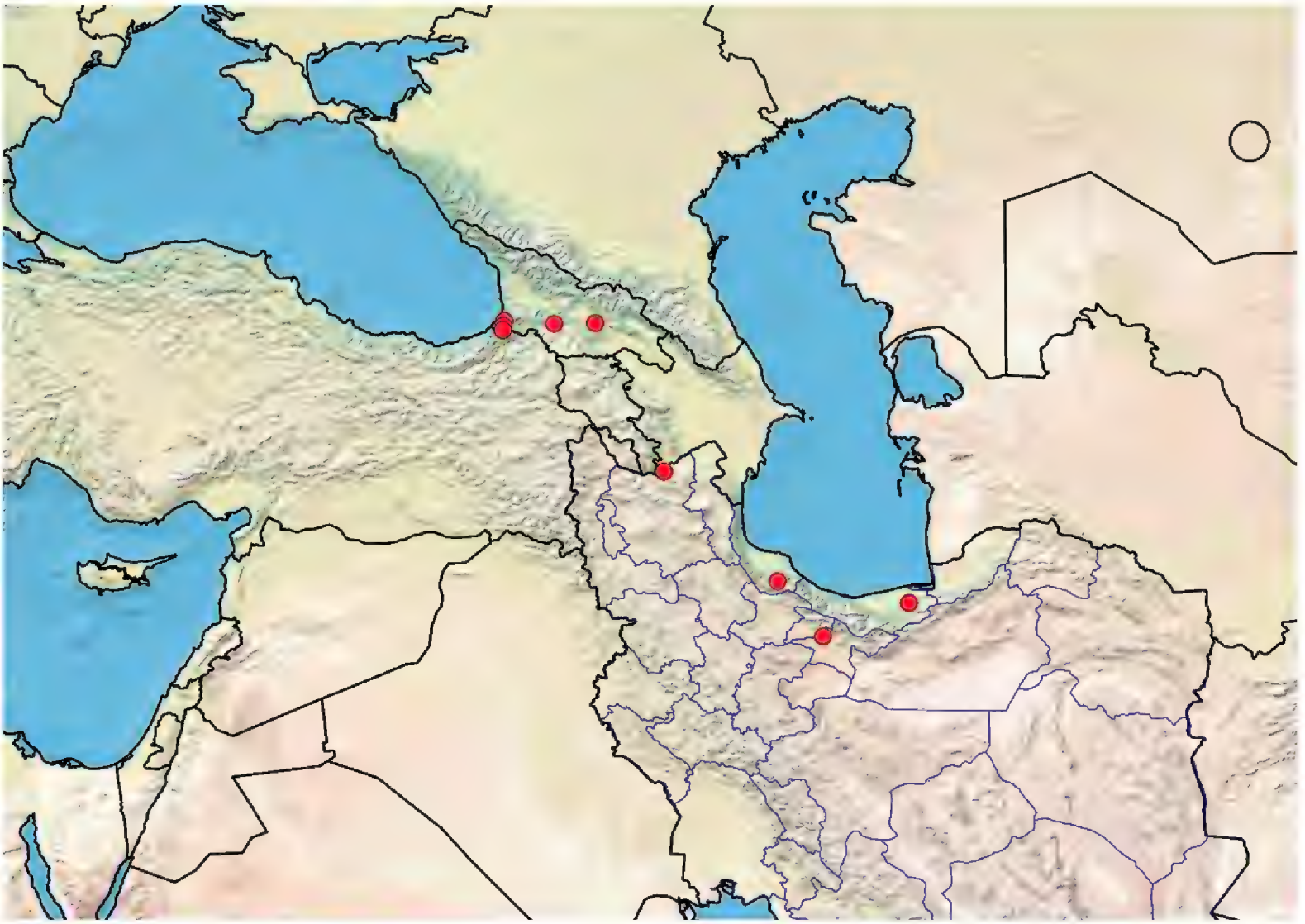

Figure 9. Map of all records of *Tischeria caucasica* in Georgia and Iran. Map prepared with QGis 3.10.

Georgia, 1 ♂ Lesser Caucasus, Samtskhe-Javakheti, Borjomi, Kvabiskhevi, 8.vii.2019, Leo Vahatalo, Anssi Vahatalo leg., LepiLED, genitalia slide EvN5274, specimen barcoded, GBRD.286, Research Collection of Anssi & Leo Vahatalo.

Leafmines most likely belonging to *T. caucasica*: Adjara AR, Chakvistavi, 41°40'37"N, 41°52'23"E, 19.ix.2018, M.V. Kozlov & V. Zverev, *Castanea sativa*, 7 mines, RMNH.INS.48084–48085 (RMNH); Adjara AR, Chikuneti, 41°34'13"N, 41°51'46"E, 26.ix.2018, M.V. Kozlov & V. Zverev, *Quercus petraea* subsp. *polycarpa*, 17 mines, RMNH.INS.48093–RMNH.INS.48095; Adjara AR, Zeda Chkhutuneti, 41°28'57"N, 41°51'46"E, 23.ix.2018, M.V. Kozlov & V. Zverev, *Castanea sativa*, 1 larva RMNH.INS.31425 (barcoded), 5 mines, RMNH.INS.48128–48129 (RMNH).

Material examined for comparison
Tischeria ekebladella (Bjerkander, 1795)

Fig. 1C, F, G

Material examined. Belgium, Namur, Nismes, Tienne Breumont, 50.07849°N, 4.5444°E, 200 m, larva 2.x.1999, E.J. van Nieukerken & T. Jin leg., dry hill with limestone grassland, *Quercus robur*, 1 ♂ emerged 3.iv.2000, RMNH.INS.23623 (RMNH), genitalia slide EvN3623; **France,** Var, St. Maximin-le-Ste. Beaume, Camping, 1 ♂, 13.vi.1987, K.N. Nieuwland leg., RMNH.INS.15431 (RMNH); **Netherlands,** Limburg, Sevenum, 1 ♀, 4.vi.1983, G.R. Langohr leg., RMNH.INS.15428 (RMNH); **Netherlands,** Limburg, Well, 1 ♂, 2.vi.1983, G.R. Langohr leg., RMNH.INS.15429 (RMNH); **Netherlands,** Noord-Holland, Oude Schuithuis, 1 ♂, 1.vii.1981, J.C. Koster leg., RMNH.

INS.845174 (RMNH), slide only, genitalia slide JCK752; **Netherlands,** Overijssel, Denekamp, Kanaal Almelo-Nordhorn, 1 ♀, 10.viii.1984, J.C. Koster leg., RMNH.INS.845695 (RMNH) slide only, genitalia slide JCK1273; **Netherlands,** Overijssel, Diepenveen, Platvoet, W. Oord leg., 1 ♀, emerged 18.xii.1982, RMNH.INS.15430 (RMNH); **Netherlands,** Overijssel, Losser, De Tip, 1 ♀ , 14.vii.1981, J.C. Koster leg., RMNH.INS.15432 (RMNH), genitalia slide JCK0755, RMNH.INS.845177.

Distribution. Europe: Albania, Austria, Belarus, Belgium, Croatia, Czechia, Denmark, Estonia, Finland, France (including Corsica), Germany, Great Britain, Greece (including Crete), Hungary, Ireland, Italy (including Sardinia), Latvia, Lithuania, Luxemburg, Netherlands, N. Macedonia, Norway, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine; Asia: Turkey (Diškus and Puplesis 2003a; Koçak and Kemal 2009; Karsholt and van Nieukerken 2017). Former records of Caucasus (Diškus and Puplesis 2003a) probably all refer to *T. caucasica*, this may be also (partly) true for the Turkish records.

Host plants. *Castanea mollissima* Blume, *C. sativa*, *Quercus cerris* L., *Q. dalechampii* Ten., *Q. faginea* Lam., *Q. x hispanica* Lam., *Q. frainetto* Ten., *Q. ithaburensis* subsp. *macrolepis* (Kotschy) Hedge & Yalt., *Q. macranthera*, *Q. macrocarpa* Michx., *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd., *Q. robur*, *Q. robur* subsp. *pedunculiflora* (K.Koch) Menitsky, *Q. rubra* L., *Q. serrata* Murray (Diškus and Puplesis 2003a; Ellis 2021).

Tischeria ekebladoides Puplesis & Diskus, 2003

Fig. 1D

Material examined. Spain, paratype, Hispania, Andalusia, Camino de Istan, ca 200 m, 1 ♂, 6.iv.1984, E. Traugott-Olsen leg., ZMUC; Same data, 1 ♀, 9.iv.1984, E. Traugott-Olsen leg., ZMUC, genitalia slides Diškus 032, Diškus 086.

Distribution. Europe: Portugal, Spain; Northern Africa: Tunisia (Diškus and Puplesis 2003a).

Host plants. *Quercus canariensis* Willd. (= *mirbeckii* Durieu), *Q. suber* L. (Diškus and Puplesis 2003a).

Other Tischeriidae from Iran

Tischeria dodonaea Stainton, 1858 New for Iran

Fig. 10A, C

Material examined. Iran, East Āzarbāijān Prov., Kaleybar, 16.ix.2008, J. Buszko leg., *Quercus macranthera*, 1 ♂, emerged 26.iii.2009, genitalia slide EvN5288, HMIM.

Distribution. Europe: Austria, Belarus, Belgium, Czechia, Denmark, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Slovenia (new record, BOLD, see Fig. 11), Spain, Sweden, Switzerland, Ukraine; Asia: Turkey, Caucasus (Diškus and Puplesis 2003a; Gozmány 2012; Laštůvka and Laštůvka 2015; Karsholt and van Nieukerken 2017).

Host plants. *Castanea sativa*, *Quercus cerris*, *Q. faginea*, *Q. x hispanica*, *Q. ithaburensis* subsp. *macrolepis*, *Q. petraea*, *Q. pubescens*, *Q. robur* and *Q. rubra* (Diškus and Puplesis 2003a; Ellis 2021).

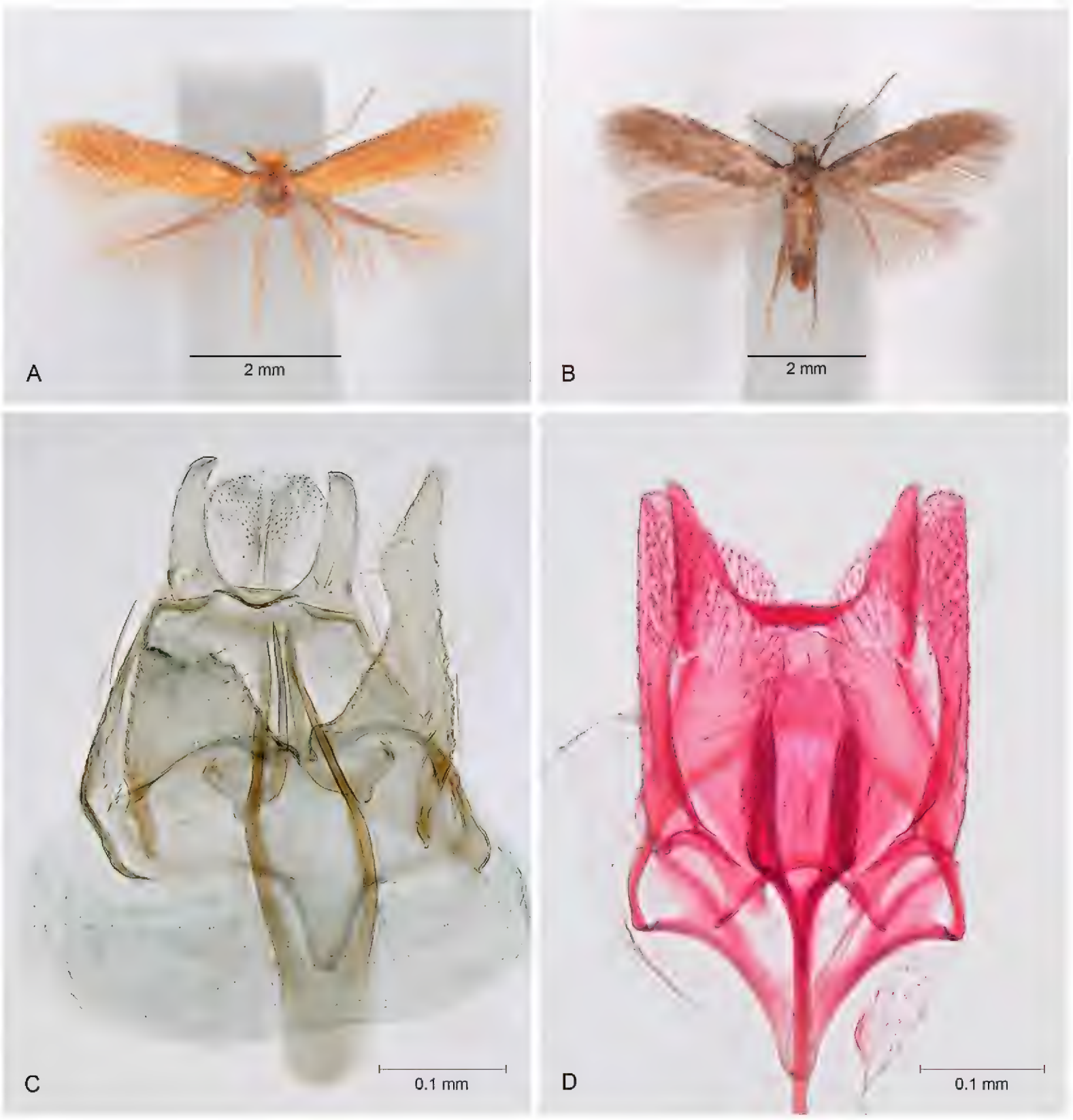

Figure 10. *Tischeria dodonaea* Stainton, 1858, East Āzarbāijān (Kaleybar), genitalia slide EvN5288 (**A, C**) and *Coptotriche gaunacella* (Duponchel, 1843), Māzandarān (Nekā), genitalia slide EvN5295 (**B, D**). **A, B.** Mounted adult males, dorsal view; **C, D.** Male genitalia.

Coptotriche angusticollella (Duponchel, [1843])

Material examined. Iran, Tehrān Prov., Tehrān, Lavāsān, 28.ix.2007, J. Buszko leg., *Rosa*, 2 mines, 1♂, 1♀, emerged 3.–8.iii.2008, genitalia Slide ♂ EvN5296, RMNH.INS.25296, RMNH, HMIM.

Distribution. Europe: Austria, Belarus, Belgium, Croatia, Czechia, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Italy (including Sardinia), Latvia, Lithuania, Malta, Moldova, Netherlands, N. Macedonia, Norway, Poland, Portugal, Romania, Russia, Slovakia,

Slovenia, Spain, Sweden, Switzerland, Ukraine; Asia: Turkey, Caucasus, Turkmenistan, far eastern Russia (Primorskiy Kray), South Korea, Japan (Honshu) (Diškus and Puplesis 2003a; Corley 2015; Aarvik et al. 2017; Karsholt and van Nieukerken 2017), and Iran (Nematollahi and Radjabi 2002).

Host plants. *Rosa* spp. (including *R. arvensis* Huds, *R. canina* L., *R. x centifolia* L., *R. x damascena* Mill., *R. gallica* L., *R. indica* L. (= *borboniana* N.H.F. Desp.), *R. luciae* Franch. & Rochebr (= *wichuraiana* Crép.), *R. multiflora* Thunb., *R. pendulina* L., *R. rubiginosa* L., *R. rugosa* Thunb., *R. sempervirens* L., *R. tomentosa* Sm., *R. virginiana* Herm.) (Diškus and Puplesis 2003a; Kobayashi et al. 2016; Ellis 2021).

Remark. The species was earlier reported from Kashan on damask rose (*Rosa damascena*) (Nematollahi and Radjabi 2002). The short description provided is confusing as they compare the moth and leafmine to a *Stigmella* species (family Nepticulidae) and also give a size of the moth that fits a *Stigmella* better; we therefore doubt that this species was correctly identified. Our current record confirms the occurrence of this species in Iran. Recently *Coptotriche angusticollella* was reported from damask rose in Turkey (Demirözer et al. 2018).

Coptotriche gaunacella (Duponchel, 1843) New for Iran.

Fig. 10B, D

Material examined. Iran, Māzandarān Prov., Nekā, 30.ix.2007, J. Buszko leg., *Prunus cerasifera*, 1 mine, 1 ♂ emerged 23.ii.2008, EvN5295, RMNH.INS.25295, RMNH; Tehrān Prov., Tehrān, Lavāsān, 28.ix.2007, J. Buszko leg., *Prunus cerasifera*, 2 mines, 1♂, 1♀, emerged 3.–5.iii.2008, HMIM.

Distribution. Europe: Austria, Belgium, Czechia, France (including Corsica), Germany, Great Britain, Greece, Hungary, Italy, Poland, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine; Asia: Caucasus, Turkmenistan (Diškus and Puplesis 2003a; Laštůvka and Laštůvka 2015; Karsholt and van Nieukerken 2017).

Host plants. *Prunus cerasifera* Ehrh., *P. cerasus* L., *P. domestica* L., *Prunus domestica* subsp. *insititia* (L.) Bonnier & Layens, *P. microcarpa* C. A. Mey., *P. persica* (L.) Stokes, and *P. spinosa* L. (Diškus and Puplesis 2003a; Ellis 2021).

Coptotriche marginea (Haworth, 1828)

Material examined. Iran, Māzandarān Prov., Vāz, 3.x.2007, J. Buszko leg., *Rubus*, mines, rearing failed; Tehrān Prov.: Tehrān, Lavāsān, 28.ix.2007, J. Buszko leg., *Rubus*, mines, rearing failed.

Distribution. Europe: Albania, Austria, Belarus, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Finland, France (including Corsica), Germany, Great Britain, Greece (including Crete), Hungary, Ireland, Italy (including Sardinia and Sicily), Luxemburg, Malta, Netherlands, N. Macedonia, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine; North Africa: Morocco; Asia: Turkey, Turkmenistan (Diškus and Puplesis 2003a; Koçak and Kemal 2009; Zerafa 2009; Karsholt and van Nieukerken 2017), Iran (Amsel 1959, 1961; Mirzayans and Kalali 1970).

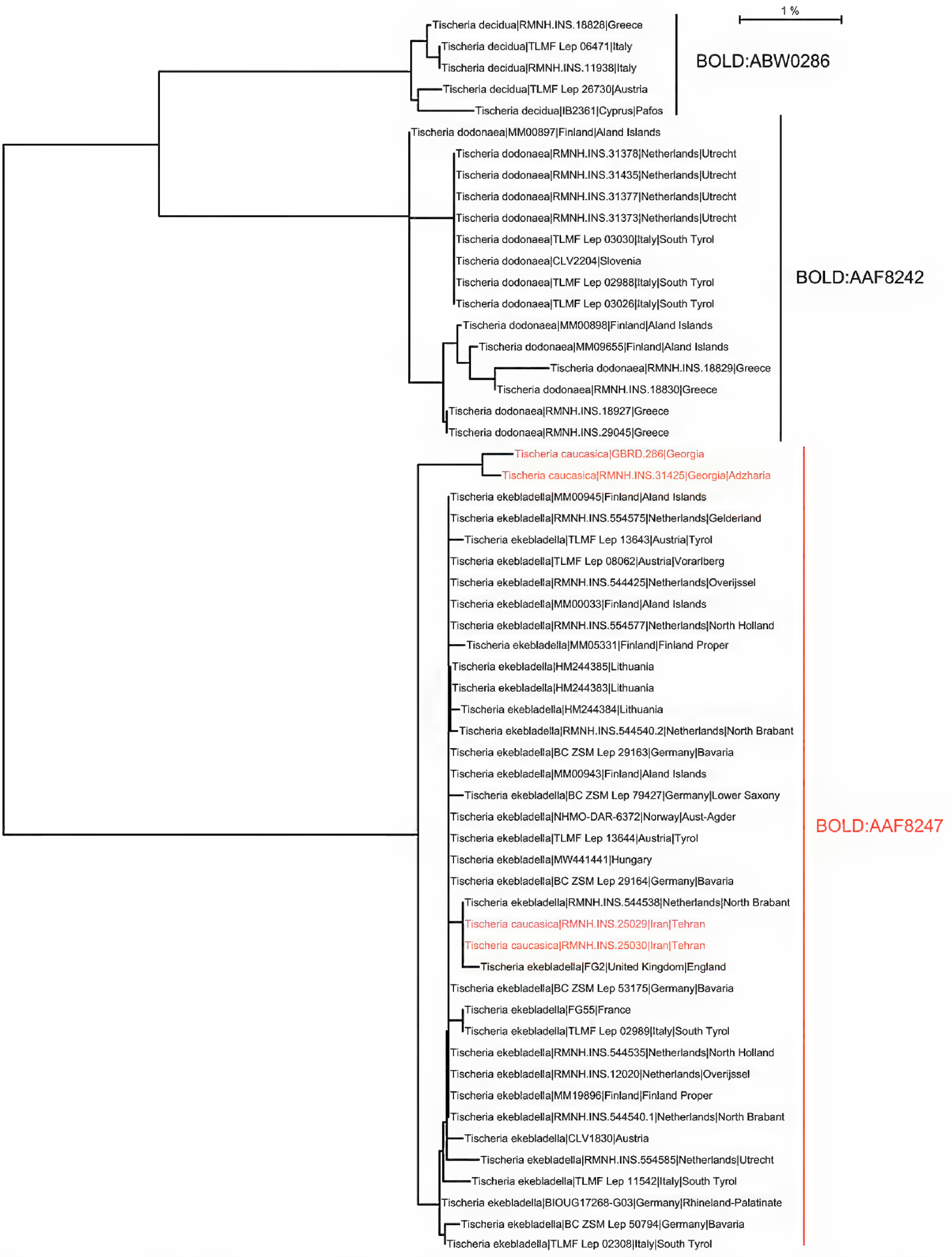

Figure 11. Neighbor Joining tree of DNA barcodes of European *Tischeria*, from dataset DS-TISCIRAN. BINs are given next to each cluster. Specimens identified as *T. caucasica* are given in red.

Host plants. *Rubus caesius* L., *R. canescens* DC., *R. fruticosus* L., *R. grabowskii* Weihe, *R. idaeus* L., *R. laciniatus* Willd., *R. macrophyllus* Weihe & Nees, *R. nemorosus* Hayne, *R. pedunculosus* D. Don, *R. plicatus* Weihe et Nees, *R. saxatilis* L., *R. tomentosus* Borkh., *R. ulmifolius* Schott (Diškus and Puplesis 2003a; Ellis 2021).

Remark. Already Christoph (1873) reported this species from Golestan, from a specimen taken around oaks; its is therefore possible that he mistook a *Tischeria* species. Later Mirzayans and Kalali (1970) recorded it from leafmines, and also our new records are based on the characteristic leafmines, as rearing failed, but confirmation by adults would still be required as several species feed on *Rubus*.

Discussion

Origin of the Tehran population

The discussed population of *Tischeria caucasica* was discovered in the National Botanical Garden of Iran in an area where planted oaks occur. All the oak species planted in the garden have Iranian origin and no oak seedlings were brought to the National Botanical Garden from other countries. However, in 2006, some oak seeds were imported from Armenia and Georgia, which were confirmed to be free of pests and diseases at that time. *Quercus robur*, the highly infected oak tree in the garden to *T. caucasica*, has been brought from the Zagros mountains at the beginning of the establishment of the garden. The seeds of this species have recently been brought to the garden for propagation and these have also been tested for pest and diseases. However, there are several gardens in Tehran with different species of planted oak trees, of which a few are close to National Botanical garden.

In Iran native oaks are limited to the northern slopes of the Elburz mountains along the Caspian Sea and the mountain range in the West (Zagros mountains), with eight species recorded (Menitskij 1984; Mozaffarian 2005; Mehrnia et al. 2013). These species include *Q. brandtii* Lindl., *Q. castaneifolia*, *Q. infectoria*, *Q. libani*, *Q. longipes* Stev., *Q. macranthera*, *Q. petraea*, and *Q. robur*. Three species, namely *Q. canariensis*, *Q. suber* and *Q. ilex* L. have been introduced as ornamentals in the country in recent years (Mozaffarian 2005). As was explained above, three *Quercus* species, i.e. *Q. robur*, *Q. infectoria* and *Q. libani* are host plants of *T. caucasica* in Tehran (Peykan Shahr). The first species occurs in the East Azarbaijan and Kordestan provinces, the second one can be found in Lorestan and most parts of Kordestan provinces, and the last species is distributed in West Azarbaijan (Mahabad), and most parts of Kordestan provinces (Mozaffarian 2005).

In conclusion, we think it is probable that this population of *T. caucasica* has entered the garden from neighboring gardens and it is overall likely that its origin is Iranian, as we also found this species on several sites in native oak forests in the north.

Status of *Tischeria caucasica* as species

When we discovered the *Tischeria* in Peykan Shahr, we were puzzled to its status as it was similar to the well known *T. ekebladella* in almost all aspects: the characteristic mines on several species of oaks, the externals and finally also the DNA barcode appeared to be inseparable. However, the juxta lobes in the male genitalia showed a diagnostic difference with *T. ekebladella*. While we were still discussing whether to describe it as a new species or not, Klasiński et al. (2020) described

T. caucasica, unaware of its biology being so similar to *T. ekebladella*. It was clear that on the basis of the characteristic juxta lobes our material belongs to the same taxon. As all specimens we examined from Iran and Georgia, from six localities far apart, show the same character of the juxta, albeit with some variability, this is a strong sign that the population from the Caucasus and Iran is a separate taxon. On the other hand, the variation of this character is hardly known for a large part of the distribution area of *T. ekebladella*, as all published figures of genitalia and all specimens examined by us are from a relatively small part of the distribution area: Northwest Europe, Sweden, Germany, Austria and Lithuania. It would be highly important to check male genitalia from the Balkans, Greece, Turkey and Russia to understand intra-and interspecific variability of male genitalia and see whether there is a clear border between the *caucasica* and *ekebladella* types or not.

Although lepidopterists tend to use genitalia characters as rather absolute identification characters, there are several cases known about variation in parts of the genitalia (Mutanen and Kaitala 2006; Mutanen et al. 2007; Goulson 2008). Evolution of genitalia differences can be very fast as response to sexual selection (Hosken and Stockley 2004; Cordero and Baixeras 2015). Whether that is here also the case requires further research.

The DNA barcodes of *T. caucasica* and *T. ekebladella* are virtually identical, where the Iranian specimens have a barcode identical to a Dutch specimen, but two Georgian barcodes group a little separate from the remaining ones (Fig. 11). More specimens need to be checked to explain this pattern; is there really a tiny barcode difference, and does the Tehran population possess European DNA barcodes due to introgression of mitochondrial DNA? The *Ectoedemia rubivora* complex of species also share the barcode, but also have very small morphological differences; here the host-plant differs and there is a small difference in a nuclear gene (van Nieukerken et al. 2012).

For now we hypothesise that *T. caucasica* is a species separate from *T. ekebladella*, without excluding completely the alternative hypothesis that the observed variation in the genitalia is intraspecific and clinal. As the barcodes of both species are almost the same, the separation must have been very recent. A fast evolution as a result of sexual selection is a possibility that deserves further study, also including nuclear genomic data.

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